



Armed Forces College of Medicine AFCM



Cardiopulmonary Module Applied Clinical Chemistry of Pulmonary Diseases

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INTENDED LEARNING OBJECTIVES (ILO)



By the end of this lecture the student will be able to:

Interpret the biochemical basis of different clinical disorders related to pulmonary system

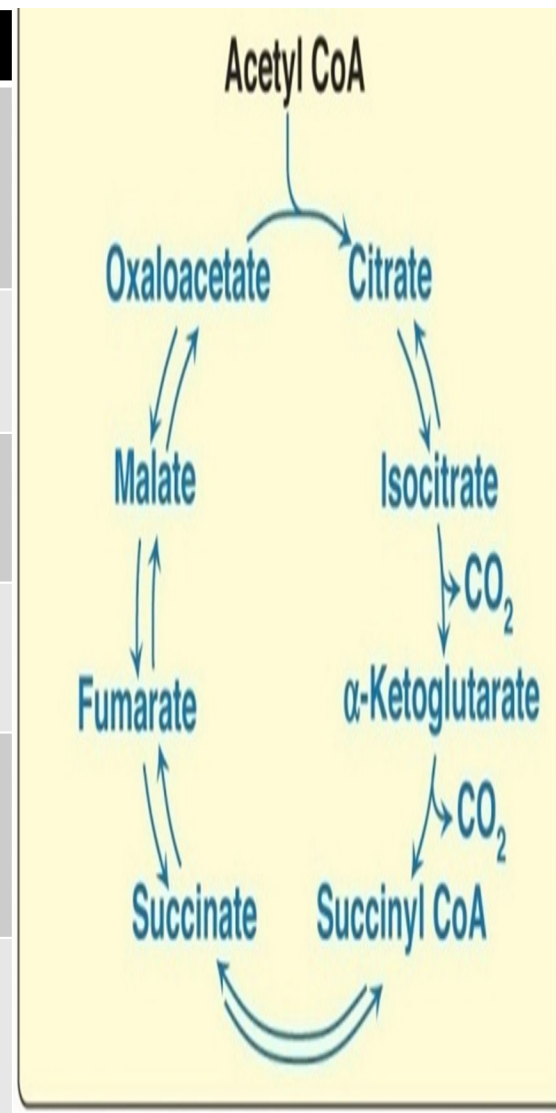


Tricarboxylic acid cycle (TCA)

**Also called Krebs Cycle
or
Citric Acid Cycle**

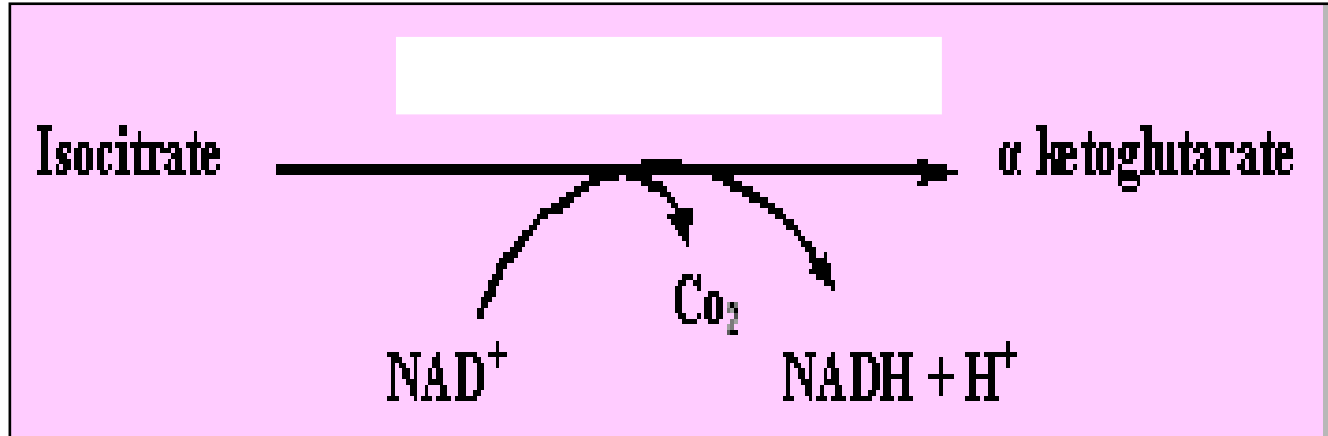
Overview of TCA cycle

Cellular location in eukaryotes	Mitochondria
Number of reactions	8
Inputs (for complete oxidation of 1 glucose molecule)	2 acetyl CoA, 6 NAD, 2 FAD, 2 ADP
Outputs (for complete oxidation of 1 glucose molecule)	4 CO₂, 2 CoA, 6 NADH, 2 FADH₂, 2 ATP
The initial acceptor	Oxaloacetate that is reformed at the end of the cycle
Key regulatory enzymes	1- Citrate Synthase 2- Isocitrate Dehydrogenase 3- α Ketoglutarate

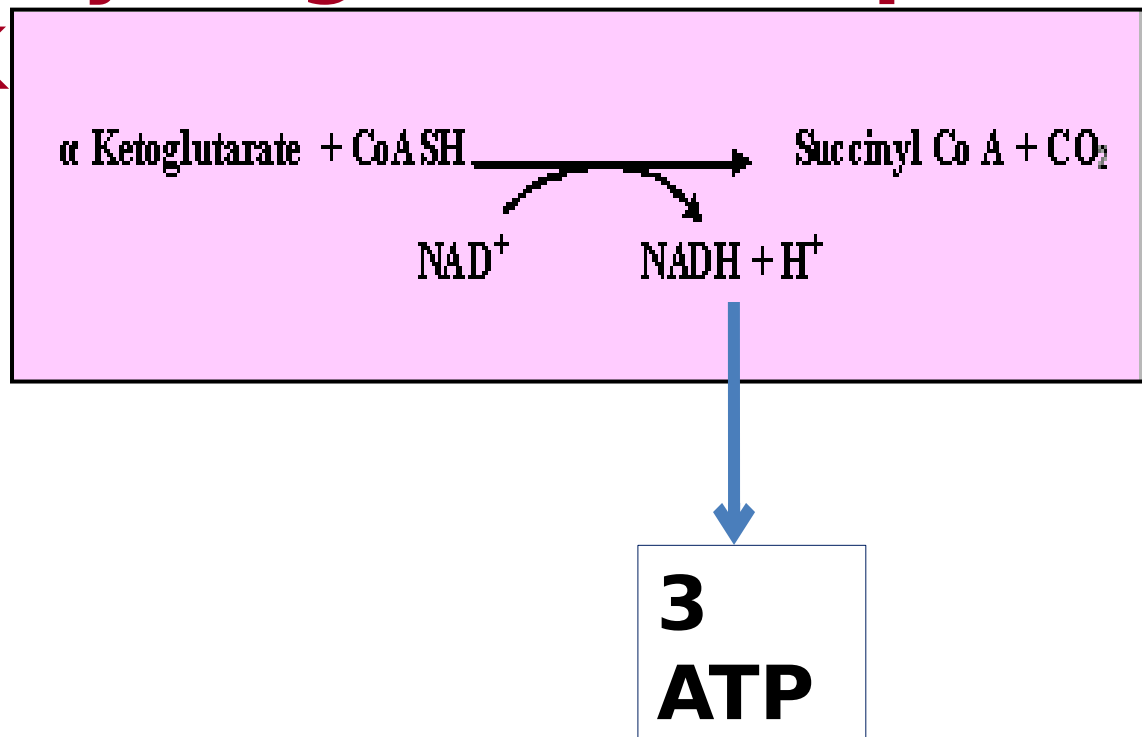


**Illustrate the
deficient parts
of the following
reaction in TCA
cycle**

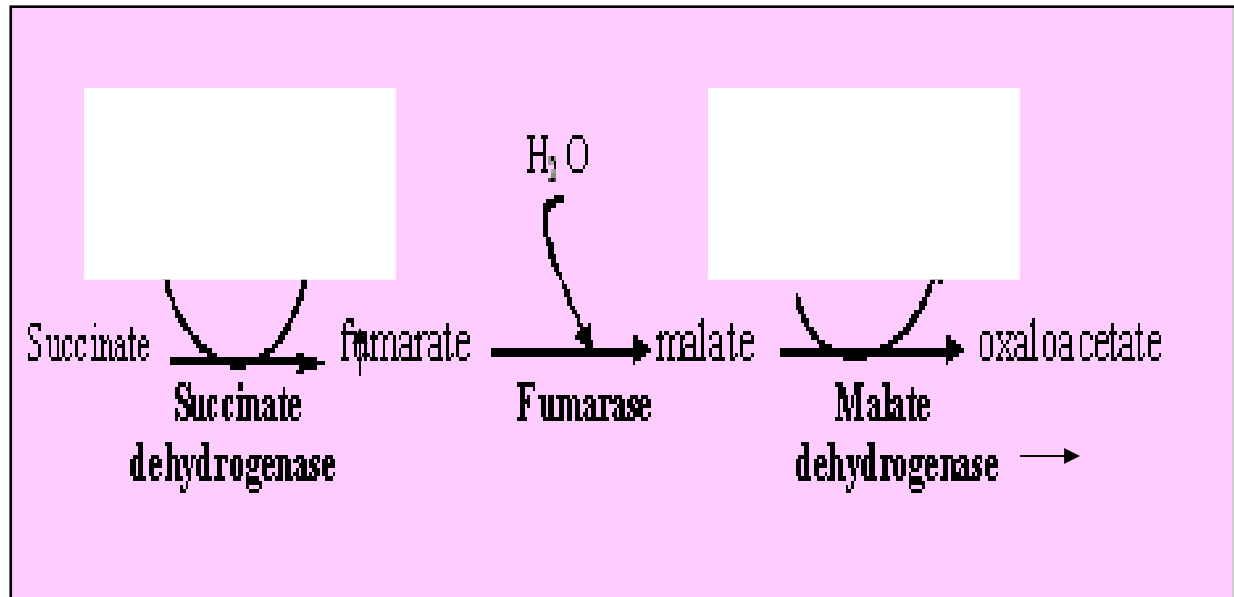
1-



2- Oxidative Decarboxylation of α Ketoglutarate to Succinyl CoA catalyzed by α Ketoglutarate dehydrogenase complex (K



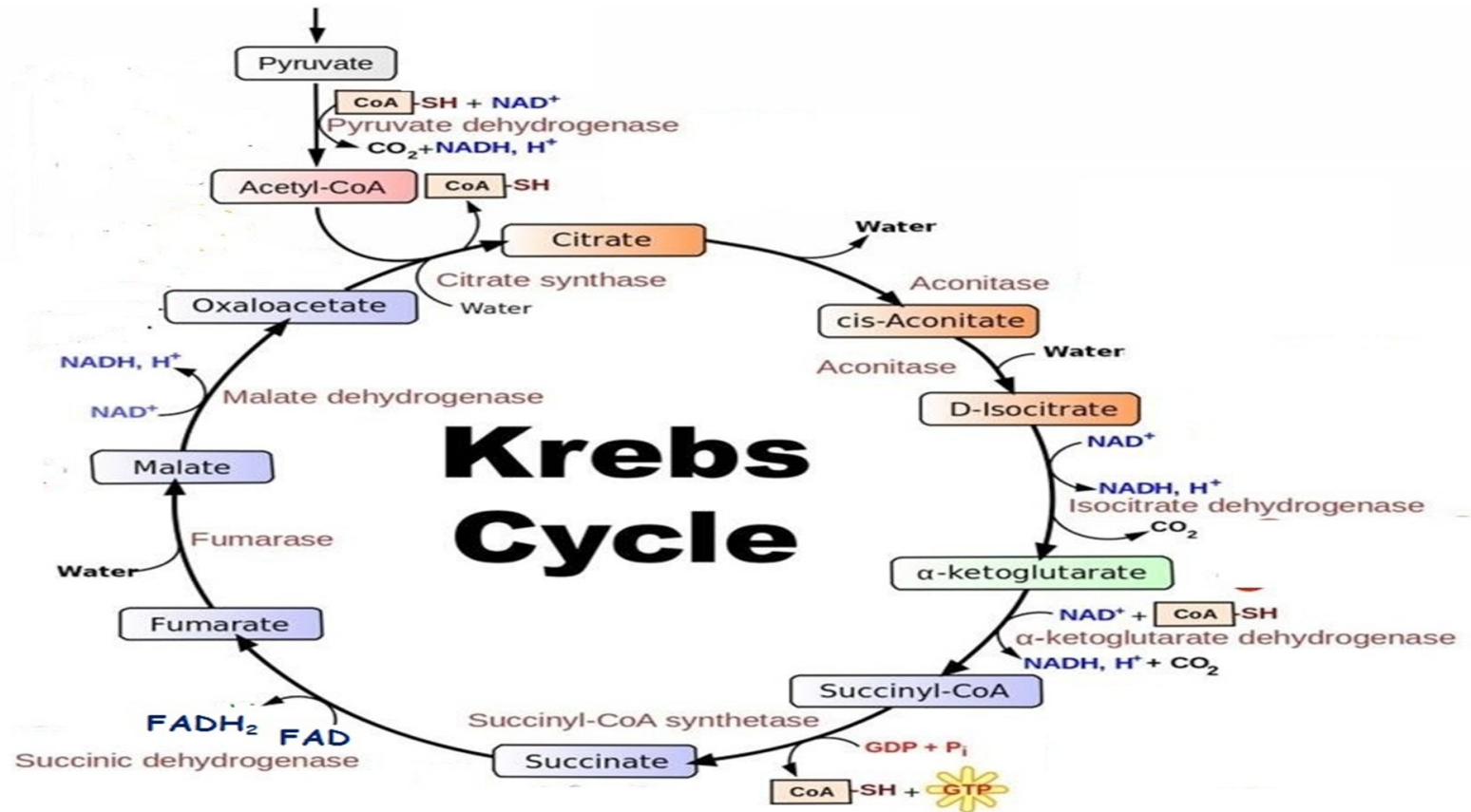
5- Oxidation of Malate to Oxaloacetate by **malate dehydrogenase**



4 members of the vit B-complex play an important role in the TCA cycle (as coenzymes).....**Illustrate them**

- **Thiamin → TPP**
- **Flavin → FAD**
- **Niacin → NAD**
- **Pantothenic Acid → CoASH**

Summarize energy yield of acetyl CoA oxidation via TCA??



TCA = 3 (NADH) + 1 (FADH₂) + 1 ATP

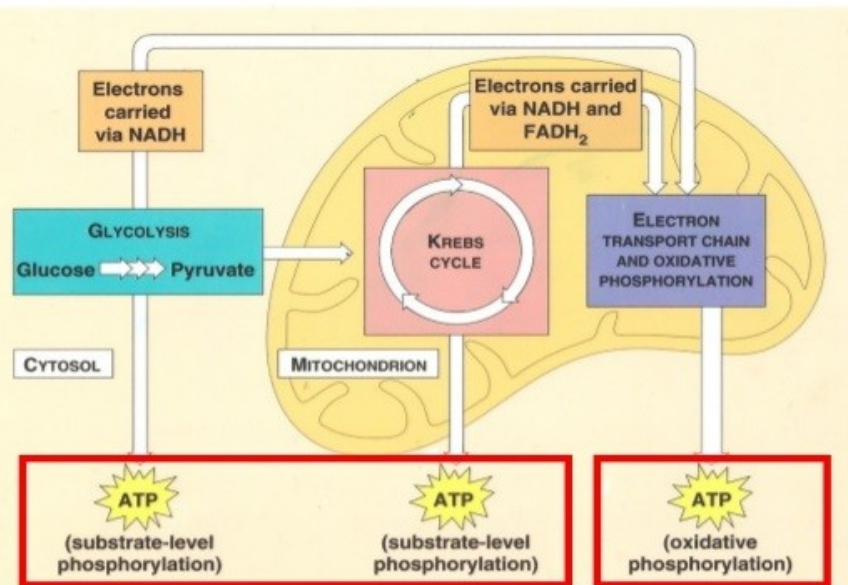
Answer the following question

Products of Krebs cycle essential for oxidative phosphorylation are _____

- (a) NADPH and ATP
- (b) Acetyl CoA
- (c) CO₂ and oxaloacetate
- ☒ (d) NADH and FADH₂

Compare between the two methods of Δ ATP synthesis

Substrate level phosphorylation	Oxidative phosphorylation
Direct ATP formation through phosphate transfer from a substrate to ADP	Indirect ATP formation from the oxidation of NADH and FADH ₂ and the pumping of electrons to generate an electrochemical gradient to make ATP
Occurs in glycolysis and krebs cycle	Occurs via the electron transport chain



NADH produced in the cytosol cannot pass through the inner mitochondrial membrane, to reach the respiratory chain and be oxidized. Shuttling of NADH is indirect by two shuttle mechanisms:

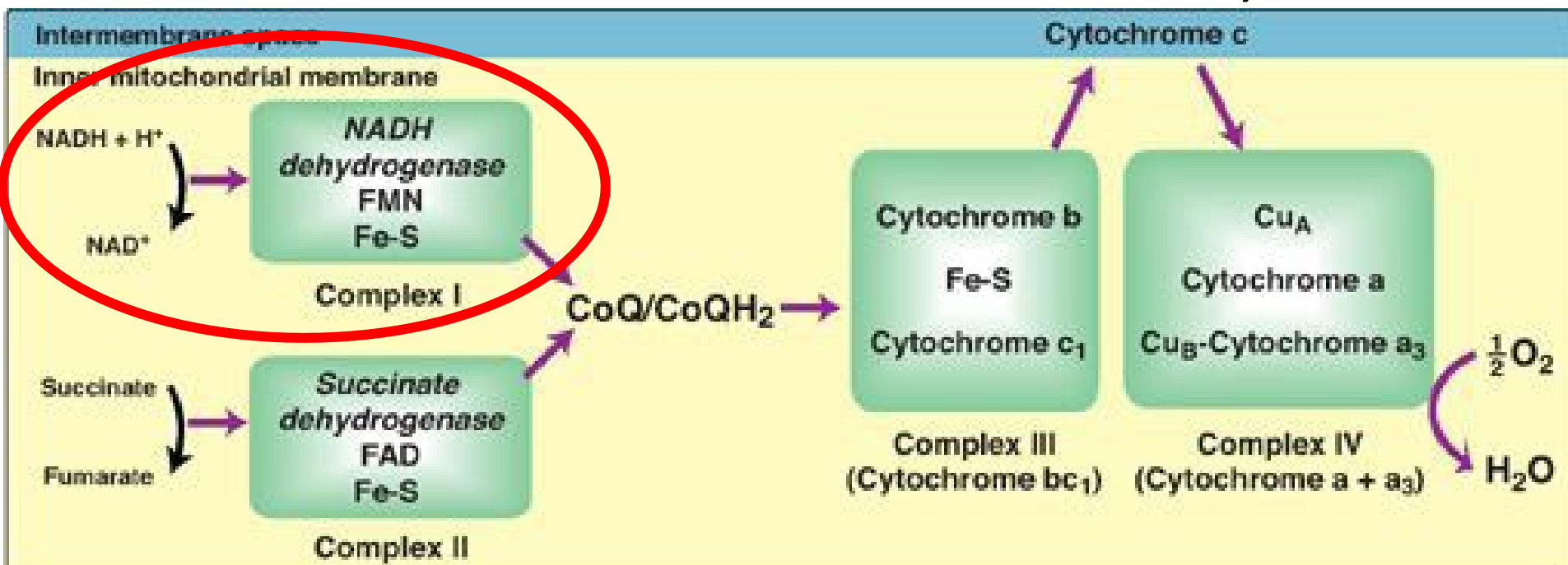
- 1- Glycerol-3P dehydrogenase shuttle
- 2- Malate-Aspartate shuttle

**What are the components of
the ETC?**

Respiratory chain components

1- Complex I [NADH dehydrogenase]:

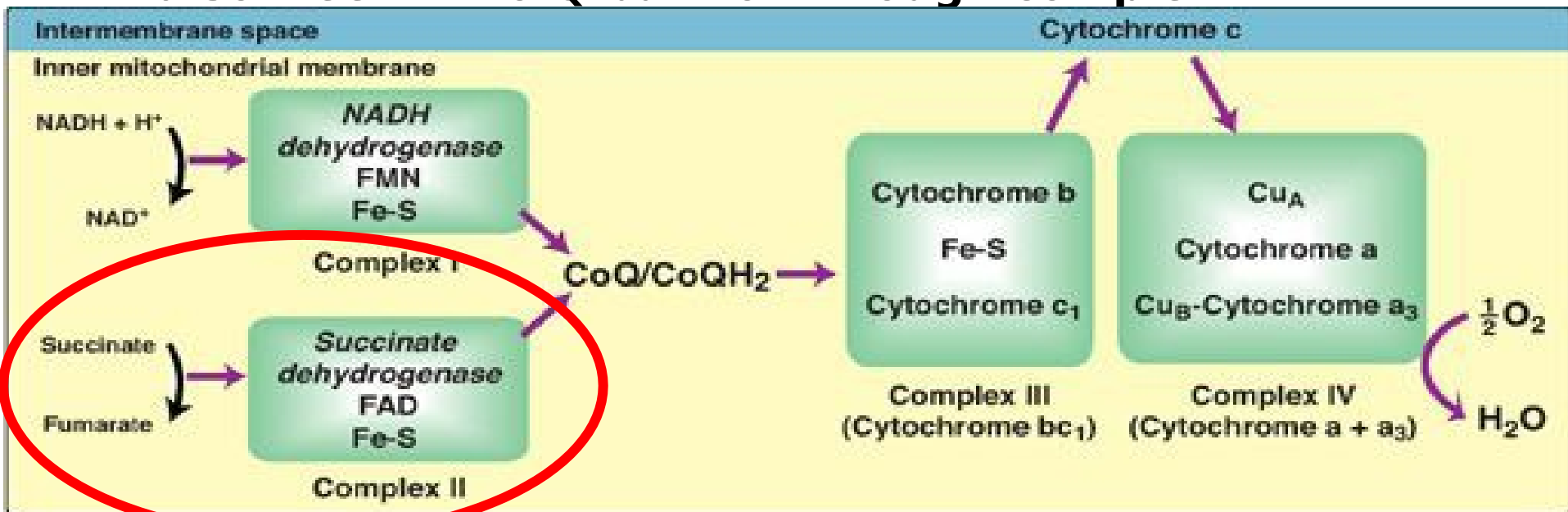
- ❖ It is a flavoprotein containing FMN & FeS as coenzymes.
- ❖ It oxidizes $\text{NADH} + \text{H}^+$ and transfer electrons through FMN and FeS to coenzyme Q which is reduced.
- ❖ During electron transfer, 2 protons (H^+) are pumped across the inner mitochondrial membrane, one ATP is



Respiratory chain components

2- Complex II (Succinate dehydrogenase):

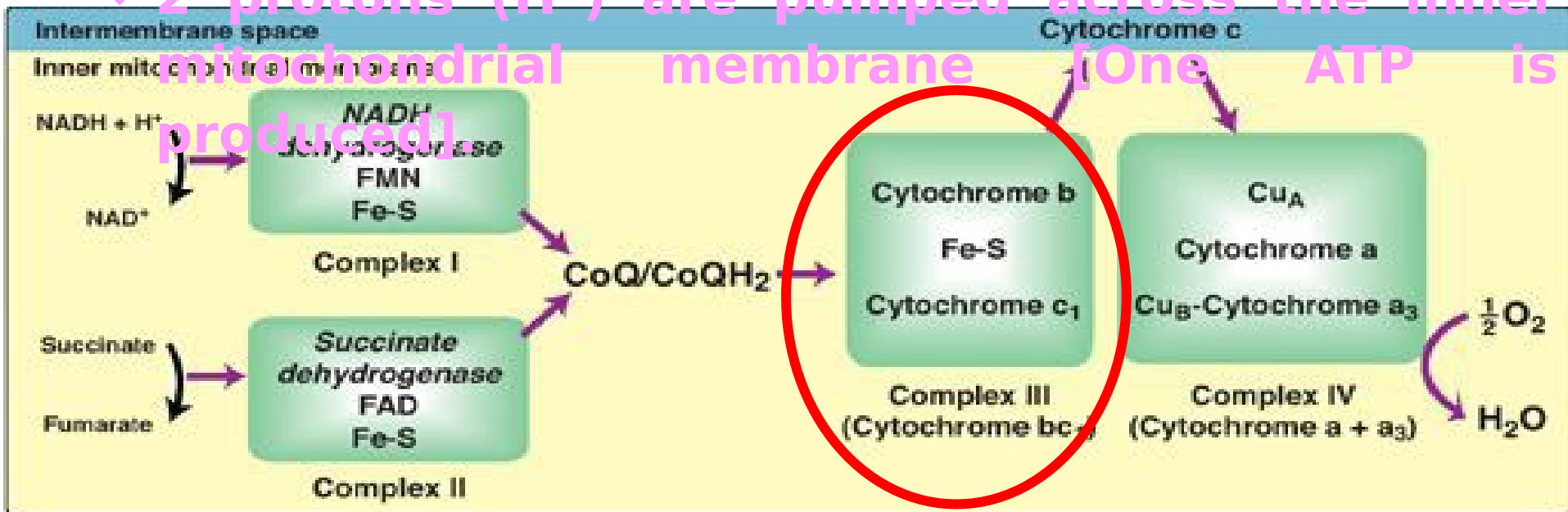
- ❖ It is the site of entry of FADH_2 .
- ❖ **Succinate dehydrogenase is the only membrane bound enzyme of TCA cycle.**
- ❖ It removes 2 H atoms from succinate to coenzyme Q.
- ❖ **No pumping of H^+ occurs at this step.**
- ❖ Glycerol 3-P dehydrogenase and acyl CoA dehydrogenase also free 2 H to Q but not through complex II.



Respiratory chain components

3- Complex III (Cytochrome c reductase):

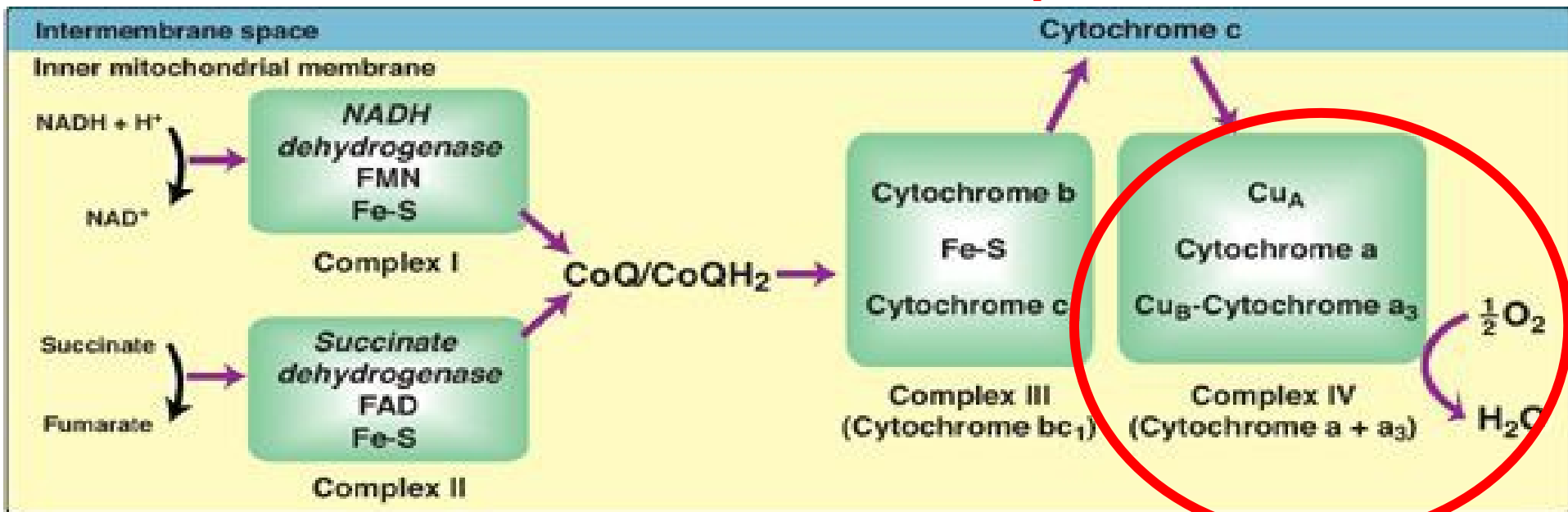
- ❖ It is composed of *Cytb* and *Cytc₁* and FeS.
- ❖ It catalyzes transfer of electrons from coenzyme Q to *Cytc*.
- ❖ 2 protons (H^+) are pumped across the inner mitochondrial membrane [One ATP is produced].



Respiratory chain components

4-Complex IV (Cytochrome c oxidase):

- ❖ It includes *Cyt a*, *Cyt a3* and 2 copper atoms.
- ❖ **Transfers electrons from red. *Cyt c* to O_2 .**
- ❖ Iron [$Fe^{+++} \rightarrow Fe^{++}$] and Cu [$Cu^{++} \rightarrow Cu^+$] are involved in oxidation/reduction.
- ❖ **2 protons (H^+) are pumped across the inner mitochondrial membrane [One ATP is produced].**



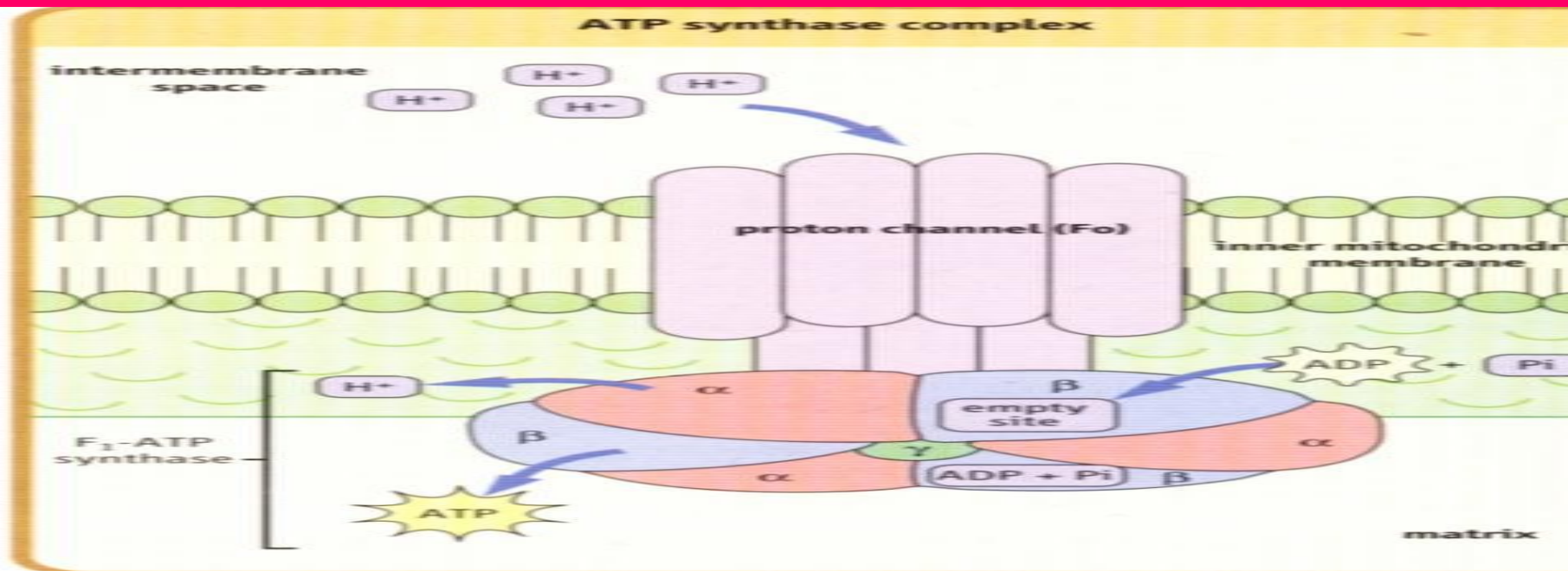
What is the mechanism of ATP synthesis through ETC?

Mechanism of ATP synthesis (**Chemiosmotic theory**)

✓ **Transfer of electrons along the ETC → outward pumping of protons across the inner mitochondrial membrane → a proton and charge gradient (electrochemical gradient) → Entry of protons through F₀ of ATP synthase → Stimulation → ATP synthesis.**

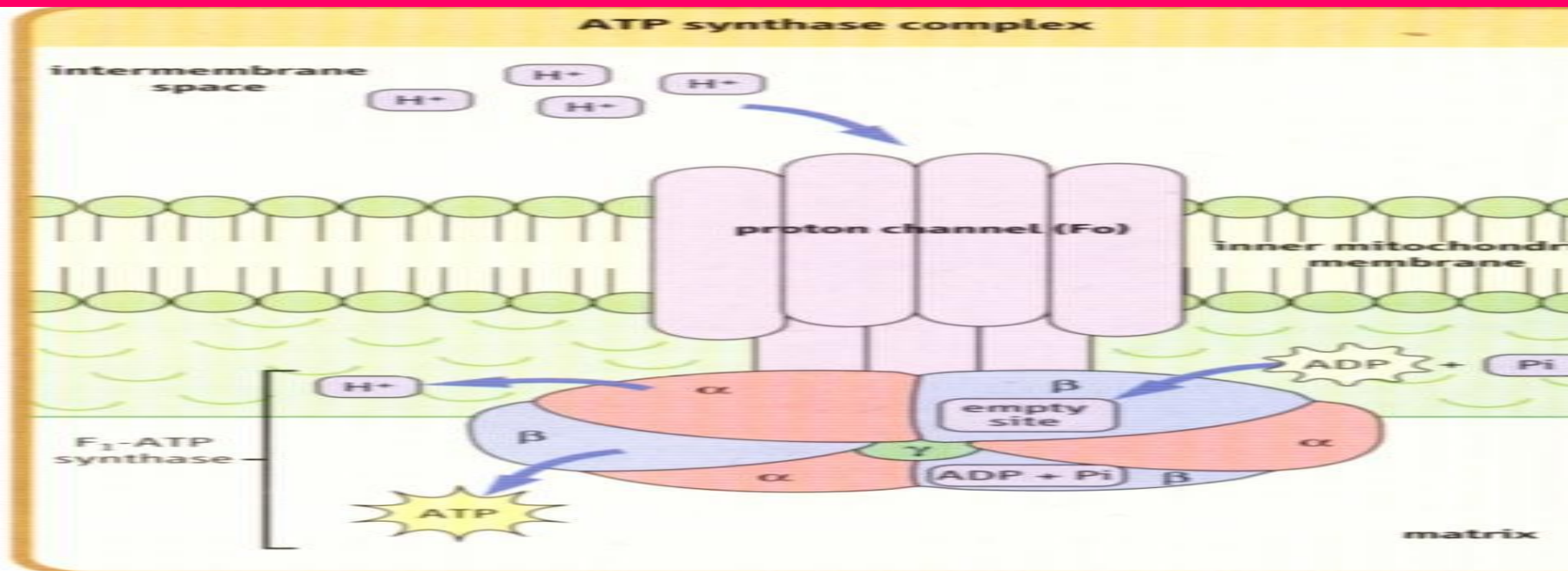
✓ ATP Synthase:

- ✓ Inner Mitochondrial membrane linked enzyme.
- ✓ Consists of several protein subunits, organized into :
 - F₀ sub-complex: discoid transmembrane, forms proton [H⁺] channel.
 - F₁ sub-complex: [3 alternating α and β subunits] which is projected into the matrix and attached to F₀ through rotatory



✓ F1 sub-complex:

- ✓ Has ATP, ADP and Pi binding sites [on β subunit].
- ✓ The flow of H through F₀ → Rotation → Activation of F₁ → Formation of ATP from ADP + Pi.
- ✓ ATP synthase only capture 68% of energy, the rest is released as heat [keeping body temp; driving the oxidation towards completion].



CASE SCENARIO

- Fumes developed at museum and several people started choking and collapsing. The authorities tell you 120 people are already pronounced dead at the scene. You noted a musty bitter almond odor when you enter the gallery.

What is the poison?



Cyanide Poisoning

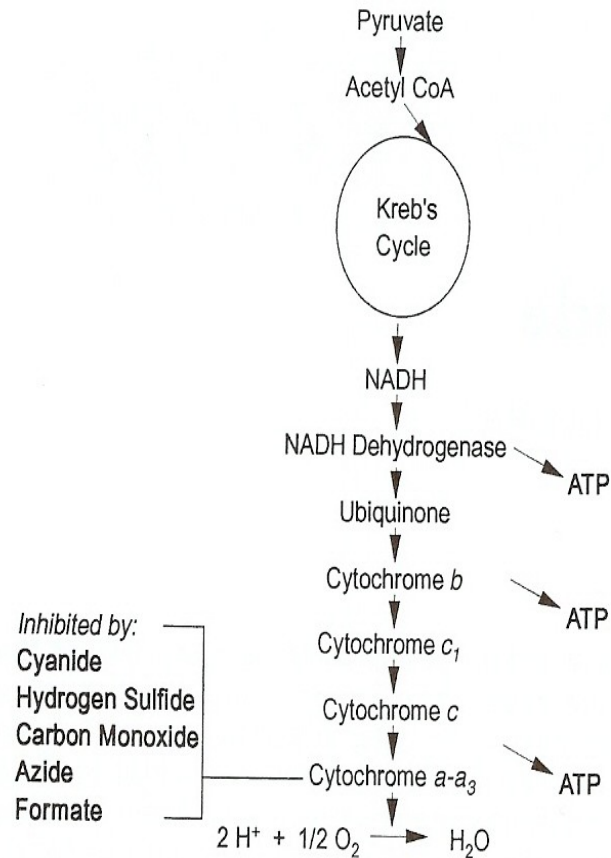


What is the
cellular
mechanism of
cyanide
poisoning?

Mechanism of action of Cyanide

- Inhibits cellular respiration
 - Cytochrome a-a3

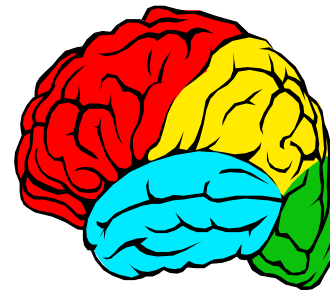
Kreb's cycle and Cytochrome



**What are the
clinical effects
of cyanide?**

Clinical Effects of Cyanide

- CNS
 - Headache
 - Dizziness
 - Seizures
 - Coma



What are the inhibitors of oxidative phosphorylation?

Comple x	Inhibitor(s)
Comple x 1	Rotenone , an insecticide binds to complex I and prevents the reduction of ubiquinone. Amytal and other barbiturates : prevent electron transfer from iron sulfur centers to ubiquinone.
Comple x 2	Malonate which acts as a competitive inhibitor of succinate.
Comple x 3	Antibiotics inhibit electron transfer through complex III by blocking transfer of electrons from Co Q to iron – sulfur proteins.
Comple x 4	Cyanide and carbon monoxide inhibit Complex IV resulting in impairment of the energy

Answer the following question

Which of the following has the highest redox potential in the respiratory chain?

- ☒ (A) O₂
- (B) Ubiquinone
- (C) NAD
- (D) FAD
- (E) FMN

A 5-year-old boy with a history of recurrent pneumonia, presented with productive cough, fever and worsening tachypnea. Physical examination revealed coarse crepitations, reduced breath sounds and clubbing. Sweat chloride analysis supported the diagnosis of cystic fibrosis. Other investigations such as bronchoscopy to look for congenital anomaly of the lung, infectious disease screening and tuberculosis, fungal and viral culture and sensitivity were negative. Further cascade screening revealed high sweat conductivity results in his siblings.

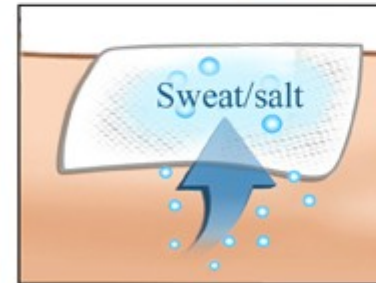
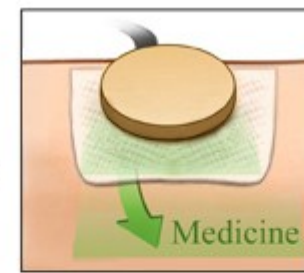
- Explain the biochemical basis of cystic fibrosis



Clubbing in cystic fibrosis

cystic fibrosis| Radiology Reference Article |
Radiopaedia.orgCystic fibrosis

A mild electrical current pushes medicine into skin to cause sweating



Sweat is collected, and salt content is measured



© Healthwise, Incorporated

Sweat chloride analysis

<https://www.nyp.org/healthlibrary/multimedia/sweat-test>

Result	Age <6 months (mEq/L Cl ⁻)	Age >6 months (mEq/L Cl ⁻)
Positive	≥60	≥60
Intermediate	30–59	40–59
Normal (CF unlikely)	<30	<40

(De Boeck et al. 2006; Farrell et al. 2008; Borowitz et al. 2009)

https://www.researchgate.net/publication/256469690_Molecular_Testing_for_Cystic_Fibrosis_Carrier_Status_Practice_Guidelines_Recommendations_of_the_National_Society_of_Genetic_Counselors/figures?lo=1&utm_source=google&utm_medium=organic

The biochemical basis of cystic fibrosis

- Defect in the gene that encodes a protein called cystic fibrosis transmembrane conductance regulator, or **CFTR**
- The most common mutation is 3 base pair deletion leading to absence of **phenylalanine** at position 508 (**$\Delta F508$**) of the CFTR protein.
- Other mutations in the CF gene produce fully processed CFTR proteins that are either non-functional or partially functional

Role of CFTR (normally)

CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR



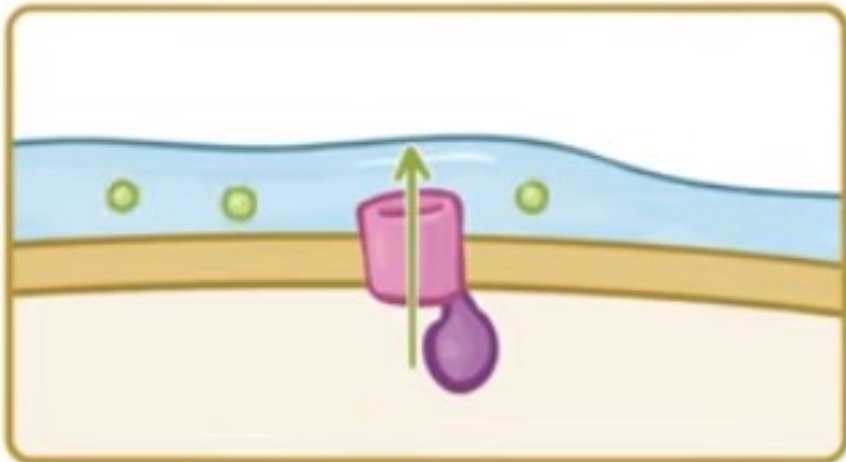
(CFTR PROTEIN)

* ATP-GATED

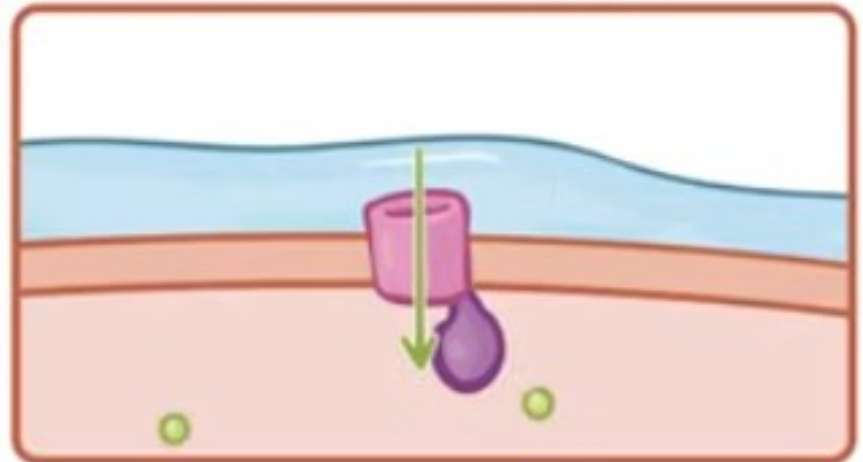
* TRANSPORTS Cl^-



MUCUS



SWEAT GLANDS



What is the laboratory evidence for CFTR dysfunction?

- 1) Two elevated sweat chloride concentrations on two separate days (>70 mEq/L)**
- 2) Identification of two CF mutations**

Answer the following question

You're educating the parents of an 8-month-old, who was recently diagnosed with cystic fibrosis, about the disease. You explain to the parents that the child has a gene mutation on the _____. The gene that is specifically mutated is called?

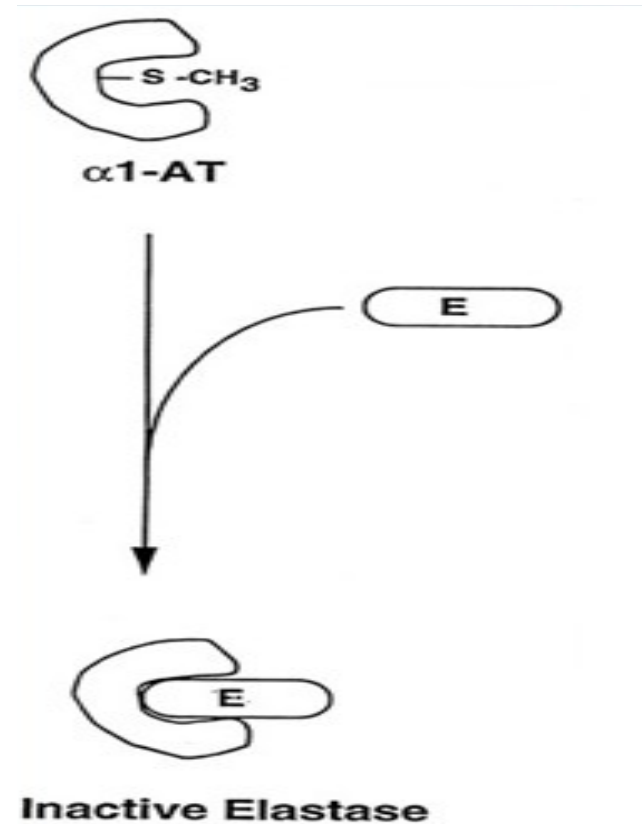
- A. endocrine glands; Hbg S gene
- B. exocrine glands; CFTR gene
- ☒ C. endocrine glands; Chromosome 21
- D. exocrine glands; HTT gene

Most Alpha-1-antitrypsin (AAT) found
in plasma is synthesized and
secreted by

- a. adrenal glands
- b. kidneys
- ☒ c. liver
- d. lung
- e. stomach

Explain the role of $\alpha 1$ antitrypsin in the lungs

It inhibits proteolytic enzymes including elastase enzyme which is secreted by neutrophils and degrades elastin of alveolar walls as well as other structural proteins in a variety of tissues



Explain the biochemical basis of α 1 antitrypsin deficiency

Results from different mutations of AAT gene causing deficiency of the protein

The most clinically widespread is one single purine base mutation (GAG to AAG, resulting in the substitution of **lysine for glutamic acid** at position 342 of the protein)

The mutation causes the normally monomeric AAT to polymerize within the endoplasmic reticulum of hepatocytes, resulting in **decreased secretion of AAT by the liver** decreasing the amount that gets to the alveoli

This causes **loss of elastase inhibition** resulting in degradation of elastin of alveolar walls inducing a disease called **emphysema**



Normal alveoli



Destroyed alveoli

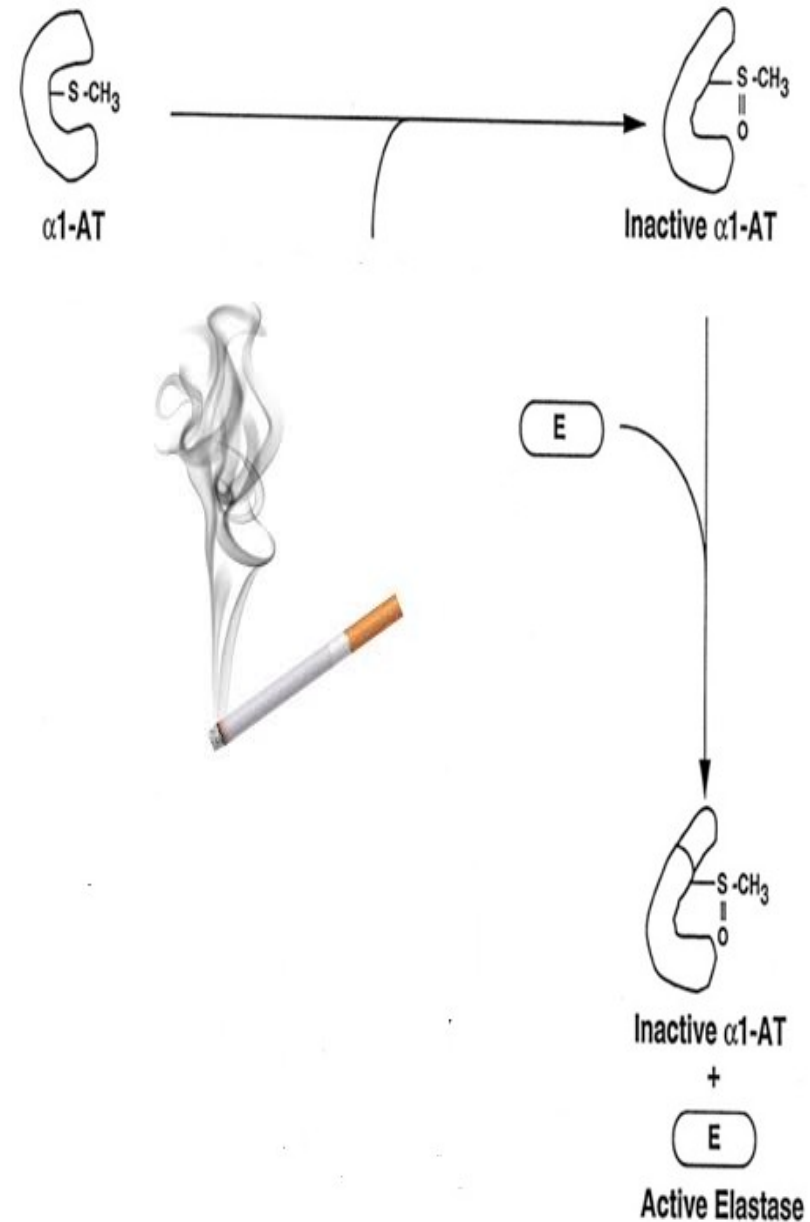
On biochemical basis explain
Smokers with AAT deficiency
have a poorer survival rate than
nonsmokers with the deficiency

- **Effect of smoking on
AAT deficiency ???**

Methionine 358 in AAT is required for the binding of the inhibitor to its target proteases.

Smoking causes the oxidation and subsequent inactivation of the methionine, thereby rendering the inhibitor powerless to neutralize elastase.

Smokers with AAT deficiency, therefore, have a considerably elevated rate of lung destruction and a poorer survival rate than nonsmokers with the deficiency.

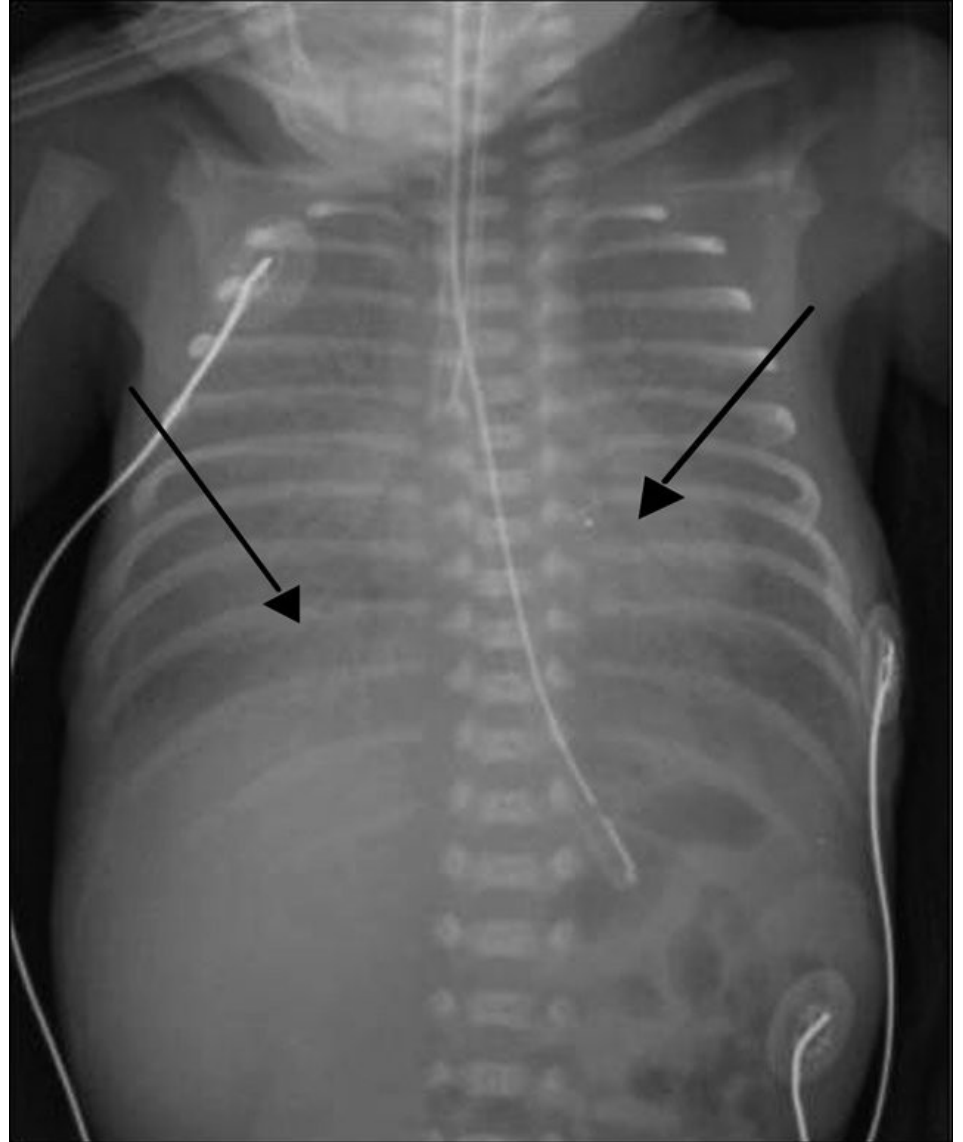


A preterm infant presents with grunting respirations appearing immediately after delivery (29 weeks of gestation), with suprasternal and subcostal retractions and flaring of the nasal alae. On examination, the baby weighs 1200 g, breath sounds are decreased, and crackles are heard. ABG is showing hypoxemia and hypercapnia; and chest x-ray shows diffuse atelectasis. A diagnosis of RDS has been established

- Explain the biochemical basis of respiratory distress syndrome



subcostal
retractions

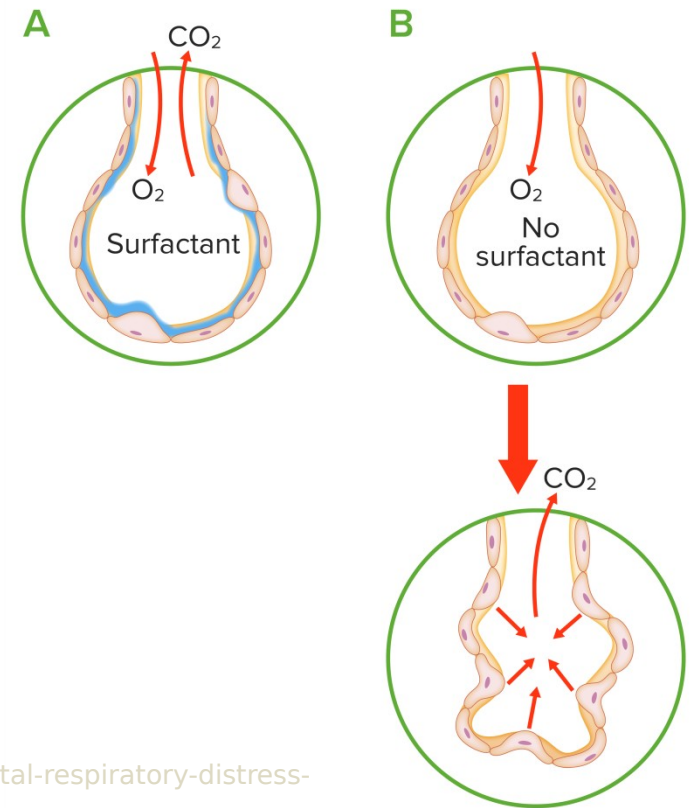


Respiratory distress syndrome. Note the "ground glass" appearance, which represents diffuse microatelectasis, and air bronchograms (arrows).

https://www.researchgate.net/publication/49650724_Isolation_Precautions/figures?lo=1

Respiratory distress syndrome (RDS)

- RDS occurs most often in babies born preterm due to lack of surfactant in the lungs.



Key points

- The **citric acid cycle** – also known as the TCA cycle (tricarboxylic acid cycle) or the Krebs cycle – is a series of chemical reactions used by all aerobic organisms to release stored energy through the oxidation of acetyl-CoA derived from carbohydrates, fats, and proteins.
- The **electron transport chain** is a series of proteins and organic molecules found in the inner membrane of the mitochondria. Electrons are passed from one member of the transport chain to another in a series of redox reactions. Energy released in these reactions is captured as a proton gradient, which is then used to make ATP in a process called **chemiosmosis**.
- Cystic fibrosis is caused by mutations in the gene encoding the CFTR protein, a Cl⁻ transporter. While Alpha-1 antitrypsin (AAT) deficiency is a genetic disorder that results from different mutations of AAT gene.

References

- "Lippincott's Illustrated Reviews in Biochemistry" by P.C.Champe, R.A.Harvey and D.R.Ferrier
- "Harper's Biochemistry" by R.K.Murray, D.K.Granner, P.A. Mayes and V.W.Rodwell.
- Fundamentals of Clinical Chemistry (Tietz) Sixth
- "Textbook of Biochemistry with Clinical Correlations" by T.M.Devlin
- **www.namrata.co- *Biochemistry for medics***

Thank

you

